THE USE OF STREPTAVIDIN COATED MAGNETIC BEADS FOR DETECTING PATHOGENIC BACTERIA BY LIGHT ADDRESSABLE POTENTIOMETRIC SENSOR (LAPS)

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ABSTRACT

A modified procedure for magnetic capture of antibody-conjugated bacteria for light addressable potentiometric sensor (LAPS) detection using the Threshold System was developed. Streptavidin coated magnetic beads, partially labeled with biotinylated anti Escherichia coli *O157 antibodies, were used to capture* Escherichia coli *O157:H7. Captured bacteria were further labeled with fluorescein-conjugated anti* -E. coli *O157:H7 antibodies and urease-labeled. anti-fluorescein antibody. Magnetically concentrated bacteria-containing complexes were then immobilized through streptavidin-biotin interactions on 0.45 μ biotinylated nitro-cellulose membranes assembled as sample sticks for the Threshold instrument. The rate of pH change associated with the production of NH_3 by the urease in urea-containing solution was measured by a LAPS incorporated in the Threshold instrument. This approach allowed us to detect 10^3 to 10^4 CFU of cultured* E. coli *O157:H7 in PBS solutions. Furthermore, detectable LAPS signals of the sample sticks remained relatively constant for at least 24 h at 4C. The developed approach was applied to detect the* E. coli *in beef hamburger spiked with the bacteria. After a 5 to 6-h enrichment at 37C, as low as 1 CFU/g of* E. coli *O157:H7 in beef hamburger could be detected.*

[1] Mention of brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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INTRODUCTION

The issue of food safety has attracted increased attention of government, industry and general public. To minimize the possibility of exposing consumers to pathogenic bacteria, their presence, if any, from farm to table, should be quickly identified and thus, proper intervention treatment may be applied. Many standard microbiological culture methods have the necessary sensitivity for pathogenic bacteria detection. However, the needs of long culture time and specialized facility remain a limit to their applications for practical purposes.

Needs to develop alternative methods for specific pathogens have been summarized in the literature (Feng 1992; Hartman *et al.* 1992). Efforts along this direction include but are not limited to ATP bioluminescence (Siragusa *et al.* 1996), antibody directed fluorescent microscopy (Tortorello and Stewart 1994), polymerase chain reaction (Bej *et al.* 1994), quartz crystal microbalance biosensor (Minunni *et al.* 1995), electrochemical enzyme immuno assays (Brewster *et al.* 1996). Culture enrichment increases cell density of targeted pathogens and thus improves probability of being detected by the approaches mentioned above. Alternatively, the cell density may be sufficiently increased by effective concentration processes. A common approach has been to use the rapid and efficient concentration by membrane filtration (Tortorello and Stewart 1994; Brewster and Mazenko 1998). The advantage of membrane concentration has been combined with the selectivity of enzyme immunoassay and the sensitivity of a light-addressable potentiometric sensor (LAPS) to the development of the commercially available Molecular Devices' Threshold System (Sunnyvale, CA). The potential of using this instrument for the detection of *E. coli* O157:H7 has been demonstrated in simple buffer systems with a detection limit as ~ 2.5×10^4 cells/mL of the bacteria (Gehring *et al.* 1998). In that work, streptavidin was used to link the bacteria which were labeled with a biotinylated anti-bacteria antibody, to the surface of biotinylated nitrocellulose (0.45 μ) membranes. However, a preliminary trial of using this process to detect the *E. coli* spiked in beef hamburger was not successful since the filter membranes were easily clogged by the particles in the meat samples (data not shown).

To circumvent this difficulty, we used immunomagnetic beads (IMB) to separate and concentrate the targeted *E. coli* from other components in the hamburger suspensions. The IMB-captured *E. coli* O157:H7 were then treated as described by Gehring *et al.* (1998) and collected on the surface of nitrocellulose membranes before detected by the LAPS (Tu *et al.* 1999). The use of this approach allowed a detection of about 1 CFU *E. coli* O157:H7 spiked in 25 g of beef hamburger after an enrichment of 6 h at 37C. While the developed process was applicable to the meat system, the IMB packed on the surface of the membrane could be dislodged by the movement of solution in the chamber of LAPS measurement. Thus, means to improve the quantitative reproducibility of the

process would be desirable. In this work, we used biotin-labeled anti *E. coli* O157:H7 antibodies that were placed on the surface of streptavidin-coated magnetic beads (SAMB), to capture the bacteria. The resulting conjugates were separated and concentrated from other components in solutions by a magnet. The SAMB with captured antibody-bacteria complex were then fixed on the surface of biotinylated nitrocellulose membranes before the detection of LAPS signals. The modification significantly improved both the sample stability of and LAPS sensitivity to captured *E. coli*.

MATERIAL AND METHODS

Culture of *E. coli* O157:H7

Cultures of *E. coli* O157:H7 (strain B1409, CDC, Atlanta, GA) were collected from slants and inoculated into a volume of 25 mL of brain-heart infusion (BHI) broth (Difco Inc., Detroit, MI) and incubated at 37C for 18 h with 160 rpm shaking. At the end of incubation, bacteria suspensions were immediately placed on ice to halt growth. The suspensions were diluted 100 folds and a volume of 6 μL of diluted sample was placed on a Petroff-Hausser bacteria counting slide (Hausser Scientific, Horsham, PA) with a center area of 0.2 x 0.2 mm further divided into 16 squares. Bacteria were counted in a random sampling of 5 of these squares. These values were averaged to determine total cell count. Cells were diluted in sterile TBS and plated either on BHI media with agar or plates restrictive for *E. coli* O157:H7 (MaConkey Sorbitol Agar, Sigma, St Louis) to determine CFU by plate counting after an eighteen hour incubation at 37C.

Preparations of Magnetic Beads

Commercially available IMB (10 mg in 1 mL of PBS, pH 7.4 containing 0.1% of human serum albumin and 0.02% NaN_3) coated with 48 to 180 μg or ~ 0.3 to 1.2 nmoles of anti-*E. coli* O157 antibodies (Dynal, A.S., Oslo, Norway) were diluted 1/10 in phosphate buffer (0.1M pH 7.4) before use. Streptavidin coated magnetic beads (SAMB) of Dynal with 650 to 900 pmoles a biotin binding capacity per mg of beads (~ 162 to 225 pmoles of streptavidin) were further treated with biotinylated anti *E. coli* O157 antibodies (0.1 to 10 μg/mg of beads) according to the following procedure. Suspensions of 100 μL of purchased stock SAMB, containing 1 mg beads, were diluted with 900 μL of TBS (tris buffered saline solution, pH 7.4) and magnetically concentrated by a Dynal magnetic particle concentrator (MPC) to remove azide. The supernatant was removed and the SAMB were washed two more times by PBS. Finally, the SAMB were suspended in 1 mL of TBS buffer and various amounts (0.67 pmole to 67 pmole) of biotinylated anti-*E. coli* O157 antibodies (Ab-I) were added and allowed to react

for 30 min at 22C. The nonconjugated Ab-I, if any, was removed from the suspension by washing the beads for 4-5 times with PBS pH 7.4 containing 0.1% BSA and then stored in the same buffer (SAMB-(Ab-I)). With 1 mg of SAMB, the treatment with 67 pmole of Ab-I, still retained about 65% of streptavidin free of biotin.

Capture of the Bacteria

Both IMB and SAMB-(Ab-I) were used to capture *E. coli* O157:H7 in pure culture media or beef hamburger suspensions. For beef hamburger, a volume of 225 mL of modified EC media, 25 g of beef hamburger and 1 mL of diluted *E. coli* O157:H7 suspension were mixed. The media contained 4 μg/mL of Sodium novobiocin (Sigma, St. Louis, Mo.) to minimize the growth of non-*E. coli* bacteria. The hamburger suspension was shaken at 37C at a speed of 160 rpm. Cultures were removed at different time intervals and filtered through glass wool to remove large fat globules and meat particles. To either pure culture or hamburger suspensions, calculated amounts of IMB or SAMB-(Ab-I) were added. The mixtures were placed on a rocker to gently mix the beads and the bacterial samples for 30 min at 22C. The beads together with captured bacteria were magnetically collected by the use of a MPC. The supernatant was removed, and the beads were washed three more times with the buffer. After the final wash, the beads were resuspended in appropriate media for further experiments.

Enumeration of Bacteria by Fluorescent Microscopy

For fluorescence microscopic experiments, general procedures described in our previous report (Tu *et al.* 1998) were used. The bacteria, captured by the beads, were suspended in PBS buffer, pH 7.4 and treated with 1 μg/mL of 4',6-diamidino-2-phenyl-indole (DAPI). The labeled *E. coli* O157:H7 were magnetically concentrated and then resuspended in PBS buffer containing 10% glycerol. Microscope slides coated with teflon grids containing 80 square wells (2 x 2 mm) from Cell-Line Associates (Newfield, NJ) were briefly etched with 5M HF + 1M HCl to increase well volumes. A neodymium boron magnet (Edmund Scientific, Barrington, NJ) was glued to the underside of the slide at the edge of an etched well. The wells were rinsed with 1% bovine serum albumin in the TBS buffer. An aliquot of 1 μL of DAPI-labeled bacteria-captured by magnetic beads (~75,000 beads) suspensions in the glycerol containing buffer was placed in a well. A coverslip was laid on the slide (edge on well border) and briefly supported (20 s) by another coverslip opposite the magnet. This created a wedge of fluid between the well bottom and coverslip in which the beads could migrate. The cover slip was then slid out and was placed on a Nikon Diaphot inverted microscope (Garden City, NY) with a 20X UV-corrected fluorescent objective lens and the fluorescence of the eleven fields along the edge of a well closest to the magnet (one field covers

an area of 272 μ x 187 μ) was measured, field by field, by moving the slide 187 μ each step with an automatic XY stage (Ludl Electronic Products, Hawthorne, NY). A script was written to move the stage in increments and capture field images so that the entire area along the edge of the well was recorded. Iterations of this script were used to capture 77 images for the entire area of the well.

Samples were illuminated by a 100 watt mercury burner with the light passing through a Nikon DM400 UV1A cube (ex:365 ± 10 nm, 400 nm dichroic, em:>400 nm). The images were digitally captured by a Pentamax 1317 cooled CCD with 1 MHZ data transfer rate using Kodak 1400 chip (Princeton Instruments, Trenton, NJ) then processed using IPlab 3.1 software (Signal Analytics, Vienna, VA). Images were threshold to approximate the perimeter of the fluorescent bacteria. Area with fluorescent intensity higher than the threshold was used to enumerate antibody immobilized and DAPI-labeled bacteria secured by the magnetic beads. For rod-shaped *E. coli* cells with dimensions approximately as 0.5 μ (diameter) x 2.0 μ (length), the possible minimal (circular) and maximum (rectangular) area of the fluorescent image of a cell are ~ 0.2 μ^2 and 1 μ^2, respectively. Since the bound bacteria on the surface of the beads assumed random orientations, the projected fluorescent area for a bound cell should vary between the maximum and the minimal areas. Thus, an average of 0.6 μ^2 was chosen to estimate the number of captured bacteria.

LAPS Measurements with the Threshold System

The operation principle and the basic construction of the Threshold System has been described in a previous report (Gehring 1998). To use this instrument for bacterial detection, the bacteria captured by magnetic beads were further conjugated by fluorescein labeled goat anti-*E. coli* antibody and urease labeled anti-fluorescein antibody (KPL, Gaithersburg, MD) according to the instructions provided by the manufacturer of Threshold System (Molecular Devices, Sunnyvale, CA). After these labelings, SAMB-(Ab-I) captured bacteria were filtered through biotinylated 0.22 μ nitrocellulose membrane attached to the sample sticks and IMB captured *E. coli* O157:H7 were filtered through either the same type of sticks or blank sticks without biotin layers. The sticks were then inserted into the sample reader chamber for LAPS detection as described (Tu *et al.* 1999). The time courses of pH change induced by the production of NH_3 near the surface of the bacteria-IMB complex sites were recorded and the initial linear slopes, expressed as potential changes ($\mu V\ s^{-1}$) were used to calculate the intensity of LAPS signals.

RESULTS AND DISCUSSION

Modifications of Sample Treatment for LAPS Detection

The detection of *E. coli* O157:H7 by the use of LAPS technology with a Threshold System was first reported by Gehring *et al.* (1998). Freshly cultured cells of *E. coli* O157:H7 in TBS buffer (Tris buffered saline solution, pH 7.0) were assayed by the standard procedure recommended by Molecular Devices (Menlo Park, CA), the manufacturer of the instrument. Three antibodies, Ab-I (biotin-labeled anti *E.coli* O157:H7 antibody) Ab-II (fluorescein-labeled anti *E. coli* O157:H7 antibody) and Ab-III (urease-labeled anti fluorescein antibody), were first allowed to form a complex with targeted bacterial cells (Fig. 1). The complex was then filtered onto to a biotinylated nitrocellulose membrane where biotin-streptavidin chemistry bounds the beads to the membrane. The procedure worked well for the bacteria in simple buffer solutions. When applied to complex food systems such as hamburger suspensions, no satisfactory results were obtained because the membrane pores were easily clogged by contaminants in the suspensions. To overcome this difficulty, a modification of the process was designed (Fig. 1). Bacteria *E. coli* O157:H7 would be first separated and concentrated from other components in hamburger suspensions by the use of Ab-I and SAMB. Separated bacteria would be further treated with Ab-II and Ab-III to form a LAPS responsive complex. The whole complex would then be collected on the surface of biotinylated nitrocellulose membrane for LAPS measurements. The feasibility of this design for detecting *E. coli* O157:H7 in beef hamburger suspensions is described in the following sections.

Efficacy of the Modification for Detection of the *E. coli* in Buffer

In the report of Gehring *et al.* (1998), a sensitivity of detecting the presence of *E. coli* O157:H7 at concentrations of ~ 10^5 CFU/mL in TBS buffer was achieved when streptavidin was used to link the antibody-bacterium complexes to the surface of biotinylated nitrocellulose membrane. However, for the reasons mentioned before, the approach was not applicable to more complex food systems. We have developed a procedure in which the Ab-I and streptavidin-coated magnetic beads shown in Fig. 1 were substituted by immunomagnetic beads (IMB) specific for *E. coli* O157:H7 and the biotin labeled membrane was replaced by plain nitrocellulose membrane with the same pore size (Tu *et al.* 1999). The modified procedure exhibited an equivalent sensitivity to the bacteria in TBS buffer. In addition, the procedure was applicable for beef hamburger suspension. The presence of about 1 CFU of *E. coli* O157:H7 per gram of beef hamburger could be detected after a 6-h enrichment at 37C. During the development of this modified procedure, we noted that the IMB packed on the surface of the membrane could be easily dislodged when stored in buffer or by the solution in the detection chamber of the

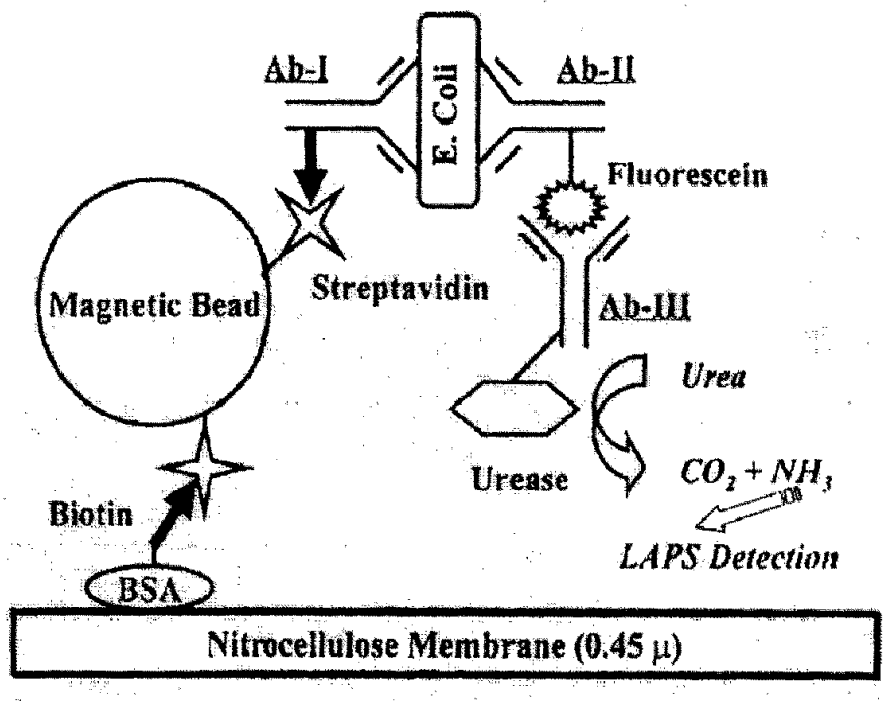


FIG. 1. IMMOBILIZATION OF MAGNETICALLY CONCENTRATED *E. COLI* O157:H7 FOR LAPS DETECTION

The details of using streptavidin coated magnetic beads to separate and concentrate *E. coli* O157:H7 captured by biotinylated anti *E. coli* O157 antibody (Ab-I) are described in text. Prior to conjugating to the nitrocellulose membrane of sample stick via biotin-streptavidin interactions, the bacteria are further treated with fluorescein labeled anti *E. coli* O157 antibody (Ab-II) and urease labeled anti fluorescein antibody (Ab-III). The potential changes induced by the production of NH_3 represent the LAPS signals.

Threshold instrument. Consequently, the LAPS signals of samples decreased rapidly as the time of equilibration between the membrane and the solution in the LAPS cells increased. This instability may be minimized by the arrangement shown in Fig. 1 where magnetic beads are tightly linked to the membrane surface via biotin-streptavidin interactions.

Samples of freshly cultured *E. coli* O157:H7 were first used to test the applicability of the arrangement shown in Fig. 1. The results shown in Fig. 2 indicate a sensitivity of detection about 10^3 CFU/mL. As shown the LAPS signal intensity is affected by the concentration of biotinylated anti-*E. coli* O157 antibody (Ab-I) used to capture the bacteria. With the concentration of the SAMB as constant, the signal increases as the concentration of Ab-I increased. The log-log plot exhibits an excellent linearity with correlation coefficient square close to 0.99. The sensitivity of the arrangement shown in Fig. 1 is about 10^3 CFU/mL, a ten fold improvement than our previous approaches (Gehring *et al.* 1998; Tu *et al.* 1999).

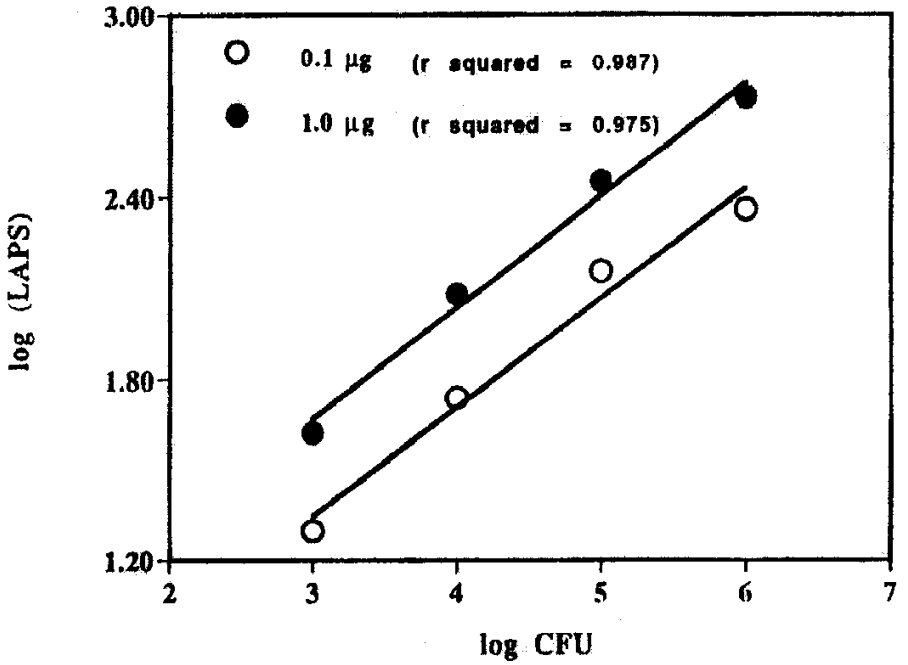


FIG. 2. SENSITIVITY OF THE LAPS METHOD IN DETECTING *E. COLI* O157:H7 IN BUFFER Freshly cultured cells of *E. coli* O157:H7 were serially diluted with PBS buffer to indicated CFU/mL. The bacteria were than captured and concentrated by the use of different concentrations of variable Ab-I but fixed concentration of SAMB and all other components needed for the LAPS detection. The open and solid circles represent data obtained with 0.1 and 1.0 µg of Ab-I applied per mg of SAMB. The data represented average of 3 independent determinations with an error of ± 10%.

Effects of Immobilizing Captured Bacteria on the Membrane

In the plan of Fig. 1, *E. coli* O157:H7 were captured by Ab-I and then linked to the magnetic beads through a biotin-streptavidin interaction. The beads were finally conjugated to the membrane via a similar biotin-streptavidin interaction. We have shown that a strong binding of magnetic beads to the membrane, as with biotin-streptavidin chemistry, is not absolutely essential for observing appreciable LAPS signals from captured *E. coli* O157:H7 (Tu *et al.* 1999). Indeed, a tight packing of the beads by mild vacuum used for filtration by the Threshold instrument of Molecular Devices, is sufficient for the signal measurements. Whether the strong binding between the streptavidin associated with the magnetic beads and the biotin on the surface of the membrane can provide any enhancement on LAPS signal is investigated here.

Experimentally, the bacterial complexes consisting of streptavidin-coated magnetic bead, Ab-I and *E. coli* O157:H7, were filtered through nitrocellulose membrane (0.45 µ) with and without the biotin coating before LAPS signal determinations. The filtration onto biotinylated membranes produced signals considerably higher (more than 100%) than those obtained with plain membranes (Fig. 3). The physical stability of the magnetic bead-membrane layer provided by

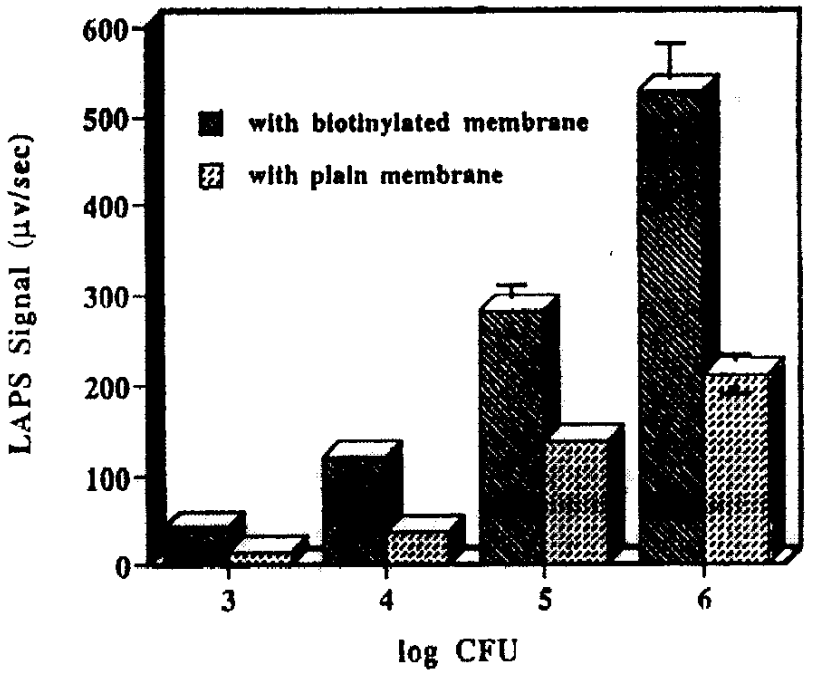


FIG. 3. EFFECTS OF IMMOBILIZING MAGNETIC BEAD-BACTERIAL COMPLEXES TO LAPS SIGNAL

Cells of freshly harvested *E. coli* O157:H7 were serially diluted to indicated CFU/mL and then captured and labeled by magnetic beads and antibodies. The complexes were then filtered through either biotinylated or plain nitrocellulose membrane sticks before LAPS measurements.

the biotin-streptavidin interactions could minimize the dislodging of the SAMB-(Ab-I)-bacteria complexes from the membrane and thus, maintained the signal intensity. This was tested and the results are summarized in Fig. 4. As shown, the samples captured by SAM-(Ab-I), after stored at 4C for 48 h, yielded a similar intensity of LAPS signals as those measured immediately after the preparation.

Comparisons Between the Use of Specific IMB and the Use of Streptavidin Coated MB

In a previous work, we reported that the use of immunomagnetic beads coated with anti *E. coli* O157 antibody for the Threshold System (Tu *et al.* 1999). The IMB method yielded a similar sensitivity as that approach of Gehring *et al.* (1998) when the bacterial suspensions in buffer were used. Thus, it is of interest to compare the sensitivities of currently developed method and previously reported IMB approach. *E. coli* O157:H7 suspensions of the same concentration were treated with either Ab-I plus SAMB or the specific IMB. After magnetic separations, both samples were suspended in buffer and further treated with Ab-II and Ab-III before being loaded on biotin coated membranes. The obtained LAPS

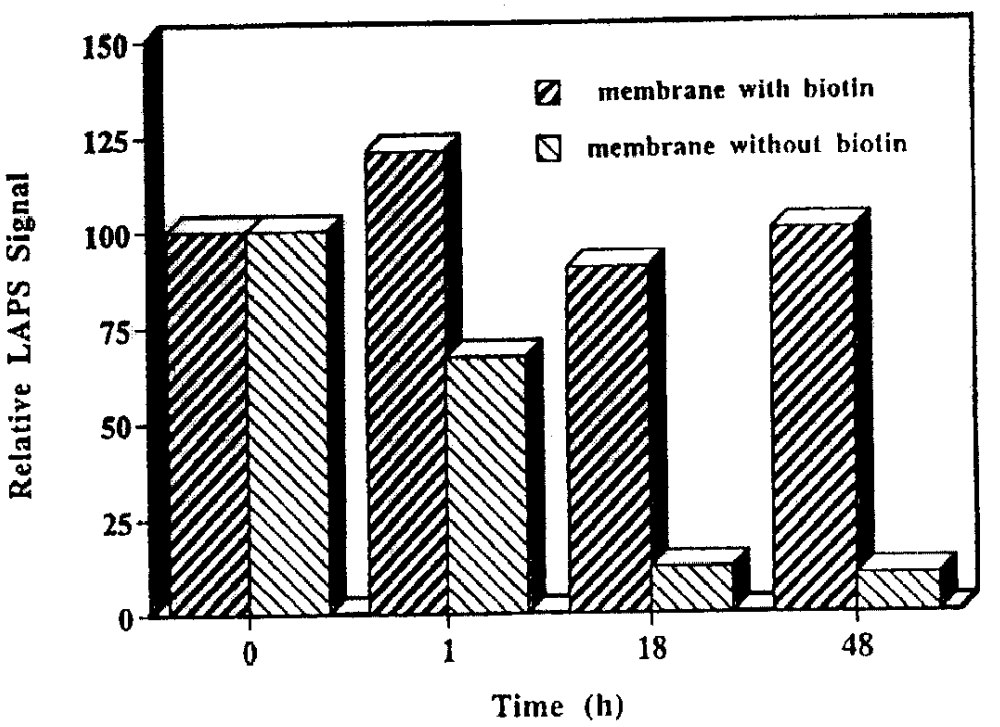


FIG. 4. STABILITY OF SAMPLE STICKS PREPARED BY SAMB-(AB-I)
Suspensions containing 10^6 CFU/mL of O157:H7 were treated with SAMB-(Ab-I) followed by further labeling with Ab-II and Ab-III. The complexes were collected on sample sticks by filtration on nitrocellulose membranes with and without biotin layers. The sticks were then stored in PBS buffer at 4C for indicated time periods before LAPS signals determined. The data represented averages of 3 independent measurements with error as of ± 10%. The 100 represents a LAPS signal of 150 $\mu V\ s^{-1}$.

results are shown in Fig. 5A. As described before, the lower signals associated with the IMB approach could be, in part, attributed to the dislodging effect of solutions used. To test the possibility that variation was due to differences in bacteria capture between two types of beads, magnetically separated samples obtained before the treatment with Ab-II and Ab-III, were treated with 4',6-diamidino-2-phenylindole (DAPI) to fluorescently label the nucleic acids in captured bacteria. Fluorescence imaging methods as described in our previous work (Tu *et al.* 1998) were then used to enumerate captured bacteria (Fig. 5B). With equal numbers of IMB and SAMB, considerably less bacteria were captured by the use of IMB. The IMB of Dynal contained 2 to 10 μg of anti- *E. coli* O157 antibodies per mg of beads. We applied 0.1 to 10 μg of Ab-I per mg of SAMB. Thus, the apparent concentrations of antibody used in both approaches were comparable. Thus, the enumeration results indicated that the combination of Ab-I and SAMB is a more efficient approach than IMB alone in capturing *E. coli* O157:H7. Therefore both the stability of captured bacteria on membrane surface and a better capture efficiency of the modified approach described in Fig. 1, contribute to the observed higher LAPS signals.

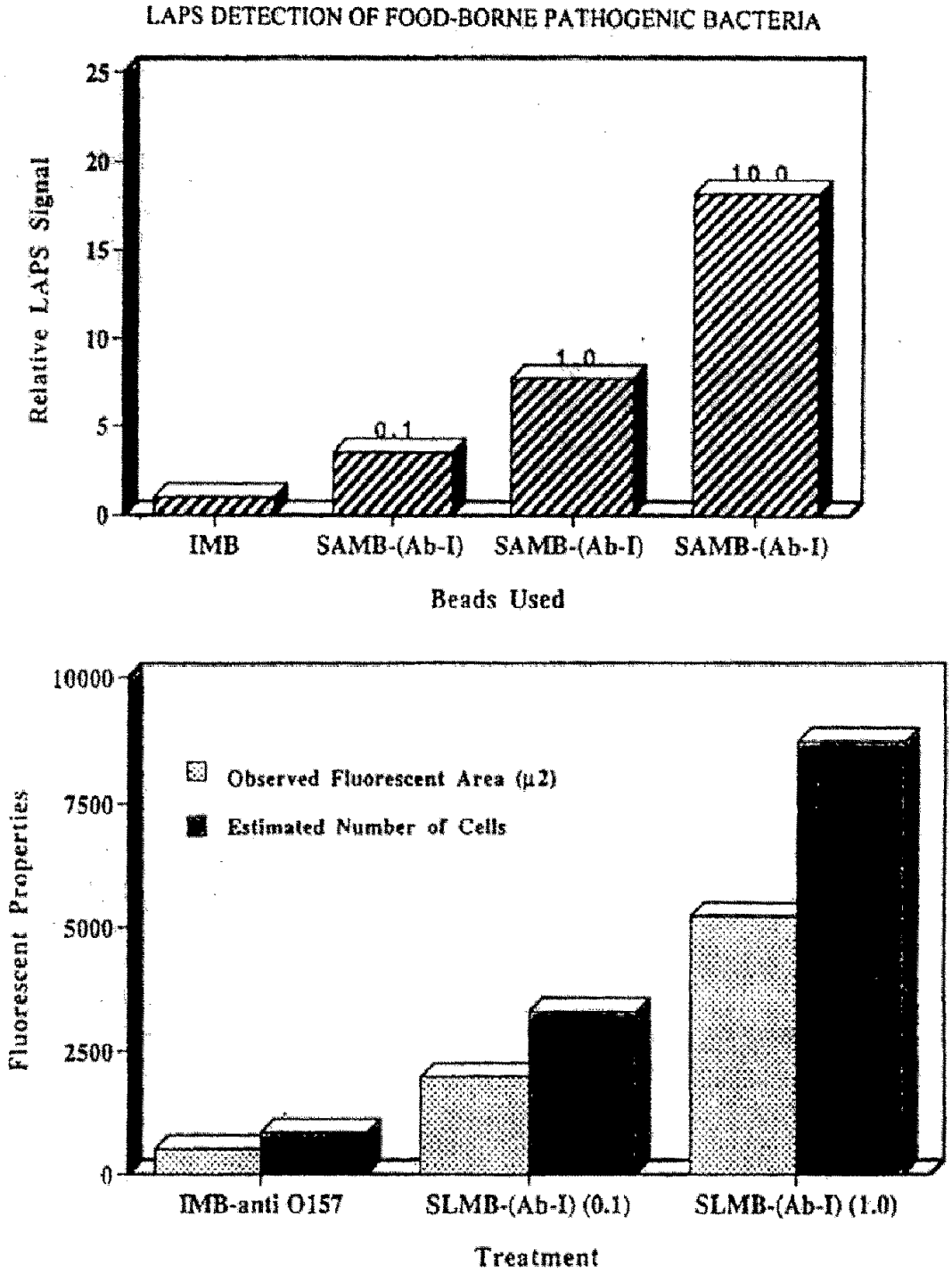


FIG. 5. COMPARISONS BETWEEN IMB AND SAMB APPROACHES

(A). *Sensitivity*. The LAPS signals obtained by the use of same number (1.2×10^6) of IMB and SAMB to PBS suspensions containing indicated concentrations of *E. coli* O157:H7. For SAMB applications, the amounts of Ab-I used were expressed in μg/mg of SAMB. After incubation at 21C for 30 min, same concentrations of Ab-II and Ab-III were added and further incubated for 15 min. The beads were then collected on streptavidin coated nitrocellulose membranes for LAPS measurements. (B) *Bacterial Capture*. Serially diluted *E. coli* O157:H7 suspensions with indicated concentrations were treated with 1.2×10^6 beads of IMB or Ab-I treated SAMB (1 μg per mg of SAMB). Captured bacteria were stained with DAPI as described in text for fluorescent microscopic imaging. Bacterial counts were obtained by dividing the total fluorescent area by the averaging cross section area of the bacteria. The data represent average of 6 experiments with error as of ± 10%.

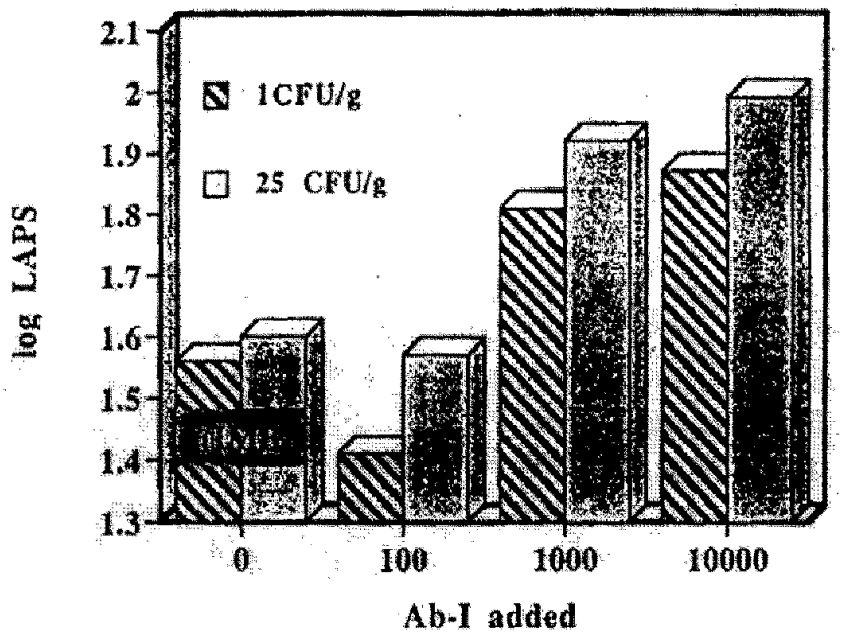
Application to Beef Hamburger Systems

The possibility of applying the described approach to more complex food system was tested by spiking beef hamburger with various levels of *E. coli* O157:H7 and/or other bacteria. The meat samples were then incubated in the proper growth media for up to 6 h at 37C. At the end of incubation, IMB or Ab-I and SAMB were added to suspensions. After separated and concentrated by the use of magnets, the magnetic beads were allowed to interact with Ab-II and Ab-III. The bacteria-magnetic beads-antibody complexes were then trapped on the sample membranes for LAPS measurements. The results shown in Fig. 6A indicated that both approaches involving the use of magnetic beads are capable of detecting low levels of *E. coli* O157:H7 after a 6-h enrichment at 37C. However, the LAPS signal intensity obtained by the use of Ab-I and SAMB exhibited an Ab-I concentration dependence. The ratios of Ab-I to SAMB for the experiments mentioned in Fig. 6A were ranging from 0.1 to 10 μg/mg of the beads. From the information provided by Dynal, there are about 3 to 10 μg of anti-*E. coli* O157 antibodies per mg (~ 6 x 10^8 particles) of the IMBs. Since the same number of IMB and SAMB-(AB-I) were used, the lower LAPS signals obtained with IMB approach suggesting a less efficient antibody-antigen interactions between the bacteria and the IMB. The exact reason for this observation is undetermined.

We have also tested the specificity of developed method toward targeted bacteria in beef hamburger systems. Beef hamburger patties spiked with *E. coli* O157:H7, or *E. coli* K-12 or both were incubated for 6 h at 37C. At the end of incubation, Ab-I and streptavidin coated MB were used to capture and concentrate the targeted bacteria for LAPS measurements. The results, shown in Fig. 6B, indicated that K-12 did not generate significant LAPS signals above than the background. Furthermore, the presence of K-12 also did not interfere the detection of *E. coli* O157:H7. This demonstrated specificity is a prerequisite for developed method to be considered as a viable alternative for detecting *E. coli* O157:H7 in foods.

CONCLUSIONS

The detection of *E. coli* O157:H7 by different LAPS techniques has been described by Gehring *et al.* (1998) and Tu *et al.* (1999). The former procedure only used membrane filtration to separate antibody conjugated bacteria from other contaminants and thus, could not be successfully applied to food systems rich in oily and fibrous particles. In the latter procedure, the physical stability of IMB-bacteria complex layer on the surface of the membrane was not adequately addressed.



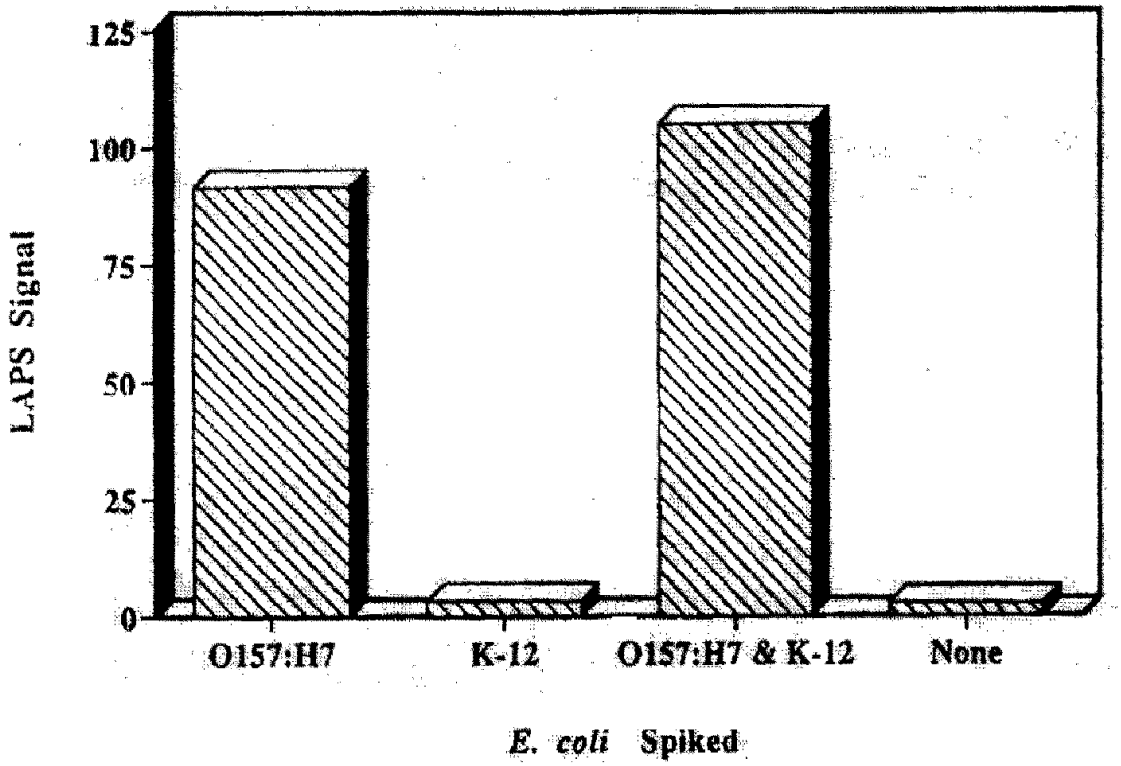


FIG. 6. APPLICATIONS TO THE CAPTURE AND DETECTION OF *E. COLI* O157:H7 IN BEEF HAMBURGER

Beef hamburger patties were purchased from local supermarkets. The meat samples were spiked with indicated levels of *E. coli* O157:H7 and enriched for 5 to 6 h. (A). LAPS signals. The bacteria in enriched suspensions were captured by 1.2 x 10^8 beads of Ab-I treated SAMB (0.1 1.0 ands 10.0 µg per mg of SAMB) or IMB. Captured bacteria were then prepared for LAPS detection as described in text. The data represent averages of 2 independent measurements with errors as ± 10%. (B) Specificity. Beef hamburger patties were spiked with indicated levels of *E. coli* O157:H7 and/or K-12 and then enriched for 5 to 6 h at 37C. Ab-I treated SAMB (1.0 µg/mg of SAMB) were applied to capture indicated bacteria for LAPS measurements. The data represent averages of 4 independent experiments with errors as ± 10%.

In current work, magnetic beads were used to facilitate the separation and concentration of *E. coli* O157:H7 from other components in beef hamburger. The magnetic bead associated bacteria-antibodies complexes were physically linked to the sample membranes through biotin-streptavidin interactions. The added stability allows bacterial sample sticks collected by the SAMB, over an extended time period, to have their LAPS signals measured at the same time. This would permit the arrangement of having a central laboratory equipped with the rather expensive Threshold System to accept sample sticks collected within in a 24 h travel radius for LAPS measurements. The method also demonstrated required specificity toward targeted bacteria (Fig. 6B). Thus, the developed method exhibited desired sensitivity, stability and specificity for a practical rapid method for detecting *E. coli* O157:H7 in beef hamburger.

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